

**Cal-590™ AM**

Catalog number: 20510, 20511, 20512  
Unit size: 5x50 ug, 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 20510)	Amount (Cat No. 20511)	Amount (Cat No. 20512)
Cal-590™ AM	Freeze (< -15 °C), Minimize light exposure	5x50 ug	10x50 ug	1 vial (1 mg)

**OVERVIEW**

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Rhod-2 is most commonly used among the red fluorescent calcium indicators. However, Rhod-2 AM is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. Cal-590™ has been developed to improve Rhod-2 cell loading and calcium response while maintaining the spectral wavelength of Rhod-2, making it compatible with TRITC/Cy3® filter set. In CHO and HEK cells Cal-590™ AM has cellular calcium response that is much more sensitive than Rhod-2 AM. The spectra of Cal-590 is well separated from those of FITC, Alexa Fluor® 488 and GFP, making it an ideal calcium probe for multiplexing intracellular assays with GFP cell lines or FITC/Alexa Fluor® 488 labeled antibodies.

**KEY PARAMETERS**
**Fluorescence microscope**

Emission	TRITC/Cy3
Excitation	TRITC/Cy3
Recommended plate	Black wall/clear bottom

**Fluorescence microplate reader**

Cutoff	570
Emission	590
Excitation	540
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

**PREPARATION OF STOCK SOLUTIONS**

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

**Cal-590™ AM Stock Solution**

1. Prepare a 2 to 5 mM stock solution of Cal-590™ AM in anhydrous DMSO.

**Note:** When reconstituted in DMSO, Cal-590™ AM is a clear, colorless solution.

**PREPARATION OF WORKING SOLUTION**
**Cal-590™ AM Working Solution**

1. On the day of the experiment, either dissolve Cal-590™ AM in DMSO or thaw an aliquot of the indicator stock solution to room

temperature.

2. Prepare a 2 to 20 µM Cal-590™ AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Cal-590™ AM at a final concentration of 4-5 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

**Note:** The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Cal-590™ AM. A variety of [Pluronic® F-127 solutions](#) can be purchased from AAT Bioquest.

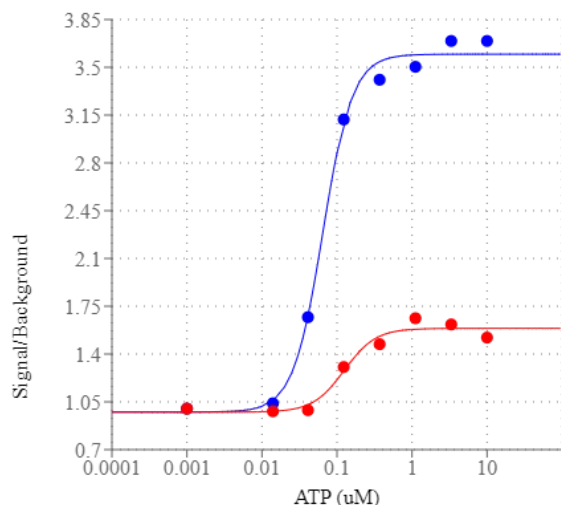
**Note:** If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of [ReadiUse™ Probenecid products](#), including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

**SAMPLE EXPERIMENTAL PROTOCOL**

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.
2. On the next day, add 1X Cal-590™ AM working solution to your cell plate.  
  
**Note:** If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.
3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.  
  
**Note:** Incubating the dye for longer than 2 hours can improve signal intensities in certain cell lines.
4. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
5. Add the stimulant as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with a TRITC/Cy3 filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 540/590 nm cutoff 570 nm.

## EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-590® AM or Rhod-2 AM under the same conditions. CHO-K1 cells were seeded overnight at 50,000 cells per 100  $\mu$ L per well in a 96-well black wall/clear bottom Costar plate. 100  $\mu$ L of 5  $\mu$ g/mL Cal-590® AM or Rhod-2 AM with 2.5 mM probenecid was added into the cells, and the cells were incubated at 37 °C for 1 hour. ATP (50  $\mu$ L/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.

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